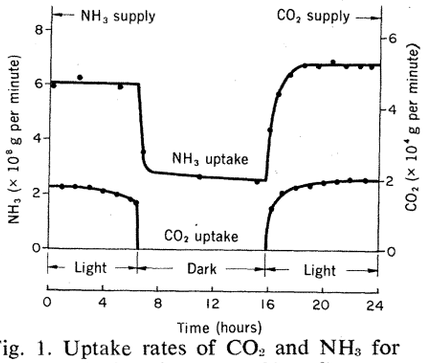

Fig. 1. Uptake rates of CO_2 and NH_3 for soybean (leaf surface area, 89 cm^2) growing in soil containing 5 mg of nitrogen as NH_4NO_3.

containing 308 g of sandy loam, 22 g of vermiculite, 33 mg of phosphorus as KH_2PO_4, 1.7 mg of iron as iron ethylenediamine di-(*o*-hydroxyphenyl acetate), 0.08 mg of boron as H_3BO_3, 3.3 mg of zinc as $ZnSO_4 \cdot 7H_2O$, 6.6 mg of sulfur as $ZnSO_4 \cdot 7H_2O$ and K_2SO_4, and variable amounts of nitrogen as NH_4NO_3. When the plants were about 15 cm tall, they were transferred to the Lucite chamber for measurement of CO_2 and NH_3 uptake rates. The uptake rates were estimated from the differences in the mass flows of CO_2 and NH_3 out of the chamber before and after the introduction of the plant, on the assumption that the composition of the gas mixture entering the chamber remained unchanged during the experiment. The total leaf surface area of the plants was estimated from tracings of the leaves on graph paper.

Data from a typical 24-hour trial are shown in Fig. 1. The NH_3 absorption rate was relatively constant during day 1 but dropped sharply at the beginning of the dark period, apparently reflecting the closing of the stomata. It is important to realize that, since the mass flow of NH_3 into the plant chamber remained constant, the lower absorption rate at night occurred in the presence of an NH_3 concentration about three times greater than the day concentration; thus the difference in day and night uptake rates is even more pronounced than is first apparent in Fig. 1. Immediately after the lights were turned on the following morning, the NH_3 absorption rate climbed rapidly and after 2 hours reached a plateau slightly higher than that which prevailed the preceding day. The higher uptake rate on day 2 is at least partially attributable to an overnight increase in the leaf surface area. The uptake of CO_2 followed a pattern similar to that of NH_3 except that the net uptake was, of course, negative during the dark period owing to the respiratory release of the gas.

The total amount of NH_3 absorbed by the soybean during the 24-hour period, about 70 μg, was nearly enough to saturate the amount of water contained in the plant, if its *p*H is 6.50. Therefore, the absence of any hint of NH_3 saturation in Fig. 1, along with the strong dependence of the uptake rate on stomatal opening, lends support to our contention that the absorbed NH_3 was metabolized rather than simply adsorbed onto exterior leaf surfaces or passively dissolved in the water bathing leaf mesophyll cells. Additional evidence is provided by Porter *et al.* (*6*), who found ^{15}N-enriched amides, amino acids, and proteins in plants previously exposed to labeled gaseous NH_3.

Table 1 compares NH_3 absorption rates for four crop species and for soybeans at three different nitrogen fertility levels. The data were taken from the absorption plateau roughly 4 to 8 hours after the initiation of the day cycle. Although the soybean plants not treated with nitrogen were visibly stunted and very chlorotic whereas those treated with 20 mg of nitrogen were very dark green and vigorous, these two classes of soybean plants showed no substantial differences in their NH_3 absorption rates on the basis of leaf area. Apparently, plants retain their capacity for absorbing NH_3 even when well supplied with nitrogen. There were some differences in the NH_3 absorption rates among species, cotton having the lowest rate and corn the highest. Although we have inadequate data for conclusive proof, we believe that these differences in absorption rates are explained entirely by species differences in internal leaf geometry, which in turn determines the resistance to diffusive transport of NH_3 across the air space inside leaves.

We believe that our data have broad implications in regard to both plant nutrition and air pollution and water pollution control. Calculations based on the data in Fig. 1 indicate that the annual NH_3 absorption by plant canopies could be about 20 kg per hectare. This rate of NH_3 supply is large enough to contribute significantly to the nitrogen budget of a growing plant community and could exert a prodigious influence on the long-term behavior of an ecosystem. Our data, together with data on the absorption of atmospheric SO_2 by plant leaves (*7*), also suggest an important role for plants in the decontamination of the earth's atmosphere.

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21 October 1971

Evidence of Pollen Tubes in Paleozoic Pteridosperms

Abstract. *A saccate pollen grain with a branched pollen tube has been discovered within the pollen chamber of a fossil seed-fern ovule of Middle Pennsylvanian age. This suggests that microgametophytes comparable to those of living gymnosperms were produced by some Paleozoic pteridosperms.*

The pteridosperms are a remarkable group of fossil gymnospermous plants with fernlike leaves, cycad-like wood anatomy, and leaf-borne pollen organs. Paleozoic pteridosperms are traditionally divided into the polystelic medullosans and the monostelic lyginopterids. During microgametophyte formation these plants produced prepollen with proximally placed trilete or monolete

sutures, and presumably proximal germination (*1*). Prepollen grains of this type are quite similar to pteridophyte spores, and are not known to have produced pollen tubes. Pollen tube production is a characteristic feature of microgametophyte development in living gymnosperms, where the position of germination is distal (*1*).

Microgametophyte development is interpreted in several plants of Paleozoic age from structures that presumably represent multicellular pollen or prepollen grains (*2*). Structures representing possible sperm have also been reported in ovules of Pennsylvanian age (*3*), but no conclusive evidence of pollen tube production is known from Paleozoic plants.

The Paleozoic fossil pollen *Vesicaspora* consists of a central body surrounded by a bilobed air bladder or saccus. The two lobes of the saccus extend toward the distal pole where they are separated by a thin slitlike area known as the distal sulcus or germinal furrow. The bladder is ornamented by an internal reticulum of inwardly directed projections. Recently, pollen assignable to the genus *Vesicaspora* has been described from pollen organs of Middle and Upper Pennsylvanian age (*4, 5*), and also from the pollen chamber of the Upper Pennsylvanian ovule *Callospermarion pusillum* (*6, 7*). The pollen organ with *Vesicaspora*-type pollen and *Callospermarion pusillum* are now considered to have been produced by the pteridosperm *Callistophyton*, of Pennsylvanian age (*5, 6*).

Ovules that conform to the genus *Callospermarion* are also present in Middle Pennsylvanian (Illinois No. 6 Coal, Carbondale formation) petrifaction material from near Carrier Mills, Williamson County, Illinois. These ovules often contain *Vesicaspora*-type pollen within the pollen chamber. One such ovule (Fig. 1A) contains a grain with a branched tubelike structure extending from the distal sulcus (Figs. 1B and 2). In the photograph, the grain is obliquely oriented and is seen in a lateral-distal view with the distal sulcus facing outward and to the right. The specimen measures 28 by 30 μm, and the somewhat deflated looking bladder exhibits reticulate ornamentation identical to previously described *Vesicaspora* grains (*8*). The tubelike structure extends from the distal sulcus (Fig. 1C) and divides twice, producing three

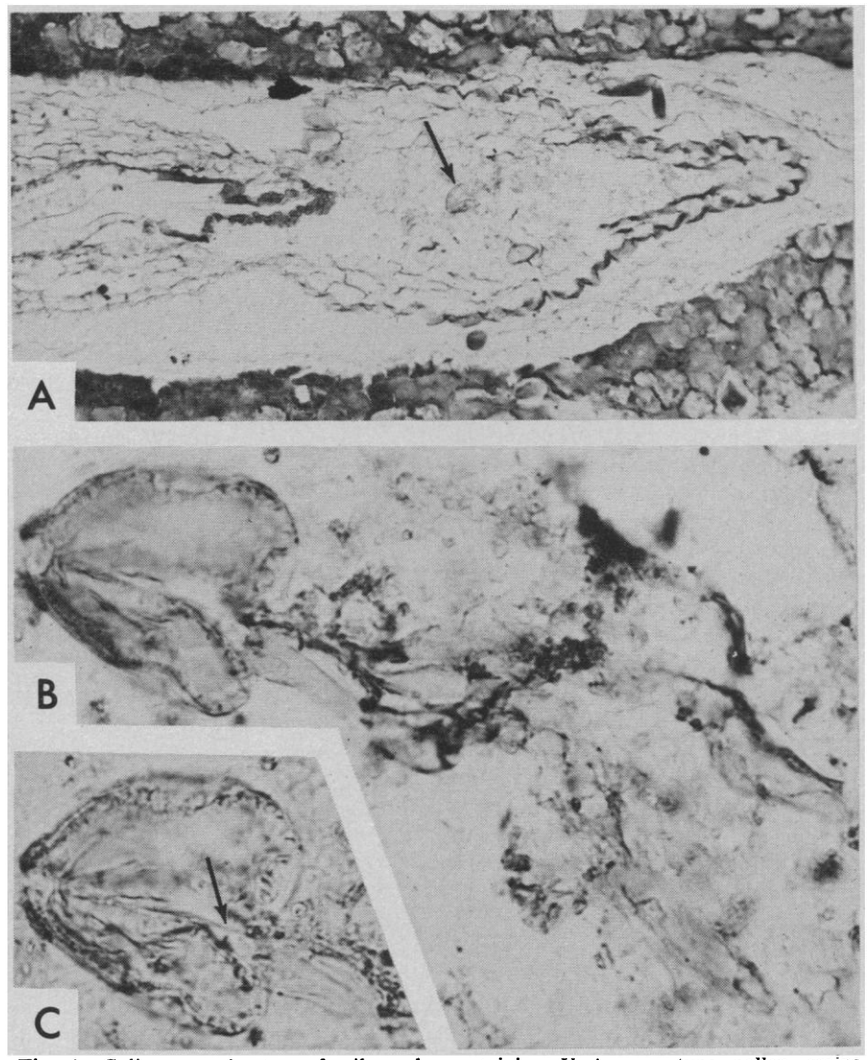

Fig. 1. *Callospermarion*-type fossil ovule containing *Vesicaspora*-type pollen grains. (A) Longitudinal section of ovule illustrating pollen chamber region with enclosed pollen grains. Arrow indicates position of grain with pollen tube (×160). (B) Pollen grain with three-lobed pollen tube (×1300). (C) Pollen grain and proximal end of pollen tube. Arrow indicates the position of tube emergence from distal sulcus of grain (×1300).

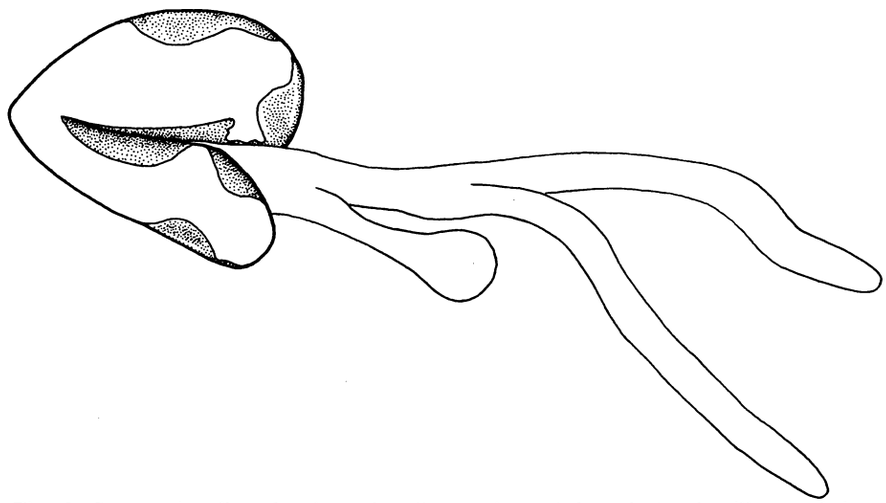

Fig. 2. Interpretive line drawing of pollen grain and branched tube illustrated in Fig. 1, B and C. Clear area in pollen grain indicates where wall was removed in the adjacent section (×1300).

slender branches (Figs. 1B and 2). Two of the branches extend approximately 70 μm and terminate in rounded tips. The third branch is shorter, measuring 21 μm long, and exhibits an inflated tip. The tube measures 7 μm in diameter at its emergence from the grain, and the individual branches each measure approximately 3 to 4 μm in diameter.

One could possibly interpret this tube as a branched, filamentous fungal hypha of the type that occasionally occurs in petrified fossil material (*9*). This latter interpretation seems improbable, however, since the tube clearly extends from the distal sulcus of the grain (Fig. 1C). In addition, there is no evidence of fungal hypha within the ovule, or in the surrounding matrix. The tube is also unlike the other contents of the pollen chamber (Fig. 1, B and C) and therefore probably is not formed by the collapsed remnants of cell walls.

Branched pollen tubes are characteristic of living cycads, *Ginkgo*, and some conifers. The structure of the *Vesicaspora*-type grain compares most closely with that of some conifers in the Pinaceae (*8*), but the pollen in this family exhibits little or no branching of pollen tubes. On the other hand, the fossil pollen tube compares more favorably with the branched pollen tubes of some species in the Araucariaceae and Taxodiaceae (*10*). The available evidence does not indicate whether this type of fossil microgametophyte achieved siphonogamy (sperm-carrying pollen tubes), or whether the tube was merely haustorial in function. The discovery of this structure does, however, indicate that pollen tubes evolved as early as the Middle Pennsylvanian, and furthermore suggests that some pteridosperms produced relatively advanced microgametophytes comparable to those of many living gymnosperms.

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23 September 1971

Cytochalasin B: Does It Affect Actin-Like Filaments?

Abstract. *An in vitro system was used to test the purported action of cytochalasin B. At concentrations 100 times those used for experiments in vivo, cytochalasin B did not cause the breakdown of F-actin, did not inhibit the transformation of G-actin to F-actin, did not inhibit the binding of heavy meromyosin to F-actin, and did not inhibit the adenosine triphosphate–induced release of heavy meromyosin from F-actin.*

Cytochalasin B (*1, 2*) "acts rather specifically in disrupting the function of contractile microfilament systems of cells" (*3*). The first evidence for such a specific effect was that cytochalasin B caused the disappearance of the 50- to 70-Å filaments of the "contractile ring" presumed to be responsible for cell cleavage (*4*). Other contractile systems which seem to function by the use of filaments have been affected by cytochalasin B, and "in every case so far examined microfilament morphology is altered by cytochalasin, and an identifiable biological process is concomitantly inhibited" (*3*).

Cytochalasin B does not affect the contraction of all contractile filament systems, however; for example, it does not seem to affect the function of smooth muscle, skeletal muscle, or cardiac muscle (*5, 6*). Cytochalasin B does not block the formation of the cleavage furrow in all cells (*1, 7–9*), and, if we assume a common "contractile ring" mechanism for furrow formation, then cytochalasin B does not "disrupt the function" of the contractile ring filaments in all cells (*8*).

To clarify the action of this compound, we studied some effects of cytochalasin B on actin filaments in vitro. Since the contractile ring filaments include actin-like filaments that bind heavy meromyosin (*10*), and since blood platelets and other systems disrupted by cytochalasin B (*3, 6, 11, 12*) contain similar heavy meromyosin binding filaments (*12–14*), then one of the specific functions being disrupted may be the actin-myosin interaction. Thus, we tested whether cytochalasin B affects the structure or function (or both) of actin.

The G-actin was prepared from rabbit skeletal muscle (*15*). The F-actin was prepared by polymerization of G-actin by the addition of 2*M* KCl to the solution of G-actin to give a final concentration of 0.1*M* KCl; after 1 hour F-actin was collected by centrifugation (at 50,000*g* for 3.5 hours). Protein concentrations of G-actin and F-actin solutions were determined by the method of Lowry *et al.* (*16*) as described by Bailey (*17*). Heavy meromyosin (HMM) was prepared from rabbit skeletal muscle (*18*). Cytochalasin B (Imperial Chemical Industries, England) was dissolved in dimethyl sulfoxide as a stock solution, the concentration of cytochalasin B being 5 mg/ml, and when used was added to the aqueous medium to give the final concentration desired.

The structure of F-actin filaments was not altered by cytochalasin B. F-actin was mixed with cytochalasin B to yield final concentrations of 0.15 mg of F-actin and 0.5 mg of cytochalasin B per milliliter of solution, the final concentration of cytochalasin B being 100 times that generally used for in vivo experiments (*1, 3, 4, 6, 8, 12*). After 1 hour, portions were placed on a grid, fixed with 1 percent formalin, negatively stained with 1 percent uranyl acetate, and observed in a Philips EM 300 electron microscope (*13*). The F-actin filaments looked normal, and thus were not altered by cytochalasin B.

Since functional properties might be "disrupted" without a change in morphology, we then made tests to determine whether the functional properties of F-actin were affected by cytochalasin B. We used HMM-binding as a measure of function. The F-actin and cytochalasin B were incubated for 30 minutes, and then HMM was added to give final concentrations of 0.15 mg of F-actin per milliliter, 0.5 mg of cytochalasin B per milliliter, and 0.3 mg of HMM per milliliter. After 15 minutes, portions were taken for negative staining (by the procedure described above). In the presence of cytochalasin B, HMM formed arrowhead complexes with F-actin (Fig. 1). These complexes looked morphologically the same as those in the controls (Fig. 2). Thus cytochalasin B did not block HMM-binding to the